EFFICACY REVIEW

PRODUCT:	89670-R, Black Pearl Paste Lodi Group. Grand Fougeray, France
DATE:	February 8, 2018
DP NUMBER:	445239
DECISION NUMBER:	474788
GLP:	No
CHEMICAL:	Alphachloralose
EPA PC CODE:	476200
PURPOSE:	Review submitted field efficacy data to determine if they support registration of the new active ingredient alphachloralose.
MRID:	50488501
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BACKGROUND:

Since early 2013, Lodi Group of Grand Fougeray, France has pursued registration of the new active ingredient alphachloralose. Two products are proposed for registration, including a technical product (89670-E) and an end-use product (89670-R) containing 4.0 or 4.45% alphachloralose.[1] As alphachloralose is not currently registered in the U.S., Lodi is in the midst of submitting the EPA-required data for a new rodenticide active ingredient for use against house mice (*Mus musculus*) indoors.

The efficacy studies required to support U.S. registration for a new active ingredient rodenticide for use against house mice are listed below.

1. A study that establishes the acute oral LD_{50} of the chemical for house mice.
2. A laboratory study that assesses the palatability and lethality of a bait containing the chemical against wild-type house mice (*Mus musculus*).
3. Five indoor field trials, each conducted in a different region of the U.S.
4. One outdoor field trial (if no claims for controlling house mice via outdoor placements is proposed, this requirement does not apply).

[1] As was noted by Bill Jacobs in a previous efficacy review dated 04/26/16, the proposed CSF and label describe different nominal amounts of active ingredient for an unknown reason.

Lodi's U.S. agents have previously expressed difficulty in getting entities to perform these tests and have proposed to reduce the number of indoor regional field trials from 5 to 3, a number upon which EPA has agreed. To date, Lodi has fulfilled the acute oral LD_{50} requirement, as well as 2 of the 3 indoor regional field trials. The data reviewed below were submitted to address the 3rd of the proposed indoor regional field trials.

DATA SUMMARY

Donahue, W. (2017) Bait Acceptance and Consumption Evaluations (Efficacy) of an Alphachloralose Rodenticide Bait Against Endemic Populations of House Mouse, *Mus musculus* Located in Field Sites in Central California. Project Number: LDI17/1. Unpublished study prepared by Sierra Research Laboratories, Inc. 90p.

MRID# 50488501

This study describes a field trial conducted at "Rainbow Farms", a poultry egg production site in Denair, CA. Donahue describes the site as a "high rise egg layer chicken operations" site "with a history of rodent infestations". The site reportedly has a long history of rodenticide use, including anticoagulant baits, with "varying degrees of success" and "no known resistance to alphachloralose". According to the report, "[this site] was chosen to conduct the evaluations due to an older bird population, i.e. greater amount of accumulated manure and high mouse pressure." The structures to be treated were "two 50' x 550' (27,500 ft^2) houses with five (5) rows of stack-caged chickens located in the second story above the fully enclosed and ventilated mature pit at ground level".

Similar to both of the previously submitted alphachloralose field trials, house mouse activity was evaluated before and after toxic baiting using census baiting, tracking scores, and live-trapping as population indices. An additional index to the mouse population, "walking visual counts", was also reportedly conducted. A trap-out phase reportedly occurred following the post-treatment census measures.[2] The sequence of events is provided below.

Pre-treatment live trapping and walking counts: 04/17/17 – 04/19/17; 04/24/17 – 04/26/17; 05/01/17 – 05/02/17
Pre-treatment census baiting: 04/19/17 – 04/20/17; 04/25/17 – 04/27/17; 05/02/17 – 05/03/17
Pre-treatment tracking scores: 04/19/17 – 04/20/17; 04/26/17 – 04/27/17; 05/02/17 – 05/03/17
Lag period: 6 days
14 days of toxic baiting: 05/09/17 – 05/23/16
Lag period: 7 days
Post-treatment live trapping and walking counts: 05/30/17 – 05/31/17; 06/05/17 – 06/06/17; 06/08/17 – 06/09/17
Post-treatment census baiting: 05/30/17 – 05/31/17; 06/06/17 – 06/07/17; 06/08/17 – 06/09/17
Post-treatment tracking scores: 05/31/17 – 06/01/17; 06/06/17 – 06/07/17; 06/08/17 – 06/09/17
Trap outs: 06/12/17 – 06/15/17

The pre- and post-treatment censuses included 3 "counts" for live trapping, census baiting, and tracking tile counts. The walking census included 2 counts for both pre- and post-treatment, temporally overlapping the live

[2] Trap-outs or "snap trapping" is not appropriate as an activity index as the traps themselves permanently remove some individuals from the possible samplable population. Instead, snap trapping is employed at the conclusion of rodenticide field trials to identify whether any residual rodent activity is occurring.

trapping censuses. While the researchers clearly made effort to run the test with all of the census methods occurring independent from one another, there was some reported overlap in the post-treatment census counts for the tracking tile counts, census baiting, and live trapping.[3]

For the pre-treatment census baiting, Detex Blocks (20-g rodenticide-free bait blocks produced by Bell Laboratories, Inc.) were placed in 30 "Protecta" mouse bait stations, with 15 stations having been used in each of the 2 poultry houses. Four Detex Blocks were reportedly placed in each bait station. After 24 hours, the amount of census bait remaining in each station was determined.[4]

Tracking activity was measured using 30, "15 by 15 cm white ceramic tiles sprayed with blue contractors chalk/alcohol", with 15 tiles placed in each poultry house. Based upon the schematics on page 14 of the report, it appears that the tracking tiles were placed randomly throughout the 2 poultry houses. Tracking scores were reportedly calculated based upon the scale below.

0 = 0% powder removed
1 = from 1 to 25% powder removed
2 = from 26 to 50% powder removed
3 = from 51 to 75% powder removed
4 = from 76 to 100% powder removed

Tracking scores were measured 24 hours after the tiles were initially placed, both for the pre- and post-treatment counts.

Live trapping was performed with the use of 10 Sherman model traps placed in each of the 2 poultry houses, with each trap baited with 1 gram of "Provoke Professional Mouse Attractant". Appropriately, numbers of mice captured for 3 live trapping days pre-treatment were compared to numbers of mice captured for 3 days post-treatment.

Walking visual counts were reportedly performed at the same time as the live trapping census counts. The walking counts were described as having been performed by "one individual researcher using a handheld clicker/tally counting the number of mice visible directly in front of them for each of the six rows [in each poultry house] while walking a steady pace with a headlamp to aid in visibility". In this way, the walking counts census method was something of a "mobile" visual counts method, otherwise similar to what is commonly performed from a stationary position for ground squirrel field trials. While the use of visual counts as a census method is somewhat unorthodox for field trials involving nocturnal species like house mice, the extremely high mouse-pressure in the poultry houses appears to have facilitated its use. As the raw data indicate that the pre-treatment counts were performed by one researcher and the post-treatment counts were performed by another, it is not clear, however, how accurate that method might have been for estimating the effects of treatment.[5]

For the 14-day toxic baiting period, 210 pre-loaded bait stations were used, with 105 stations being placed in each of the 2 poultry houses. Based upon the report, a total of

[3] This is potentially problematic in field trials because one census method may directly affect the other in a way that decreases its utility as a population census. In this instance, the mice captured in live traps on 06/08/17 would have, at least temporarily, been unable to eat census bait and/or leave track marks on that day due to captivity.
[4] With the exception of the pre-treatment census on 04/25/17 in poultry house #2, where it appears that the census bait removal was measured 48 hours after placement instead of 24 hours.
[5] The variability in how two individuals may perform visual counts is one of the reasons that visual counts performed for ground squirrel field trials are normally conducted by a single individual.

> 2,716.6 and 2,884.0 grams of Black Pearl Paste were placed into House 1 and 2, respectively. At the conclusion of the 14-day toxic baiting phase, all bait stations were removed from the houses and brought back to the laboratory for a post treatment weight s [sic] of the amount of bait remaining.

Toxic bait consumption was relatively low, with 293.9 grams of the initial placement of 2716.6 grams having been taken from house 1, and 182.5 grams of the initial placement of 2884.0 grams having been taken from house 2. Combined, only 8.5% of the toxic bait available was removed during the 14-day toxic baiting period, which was much less than what was reported in the Buczkowski 2015 field trial, and *far* less than what was reported in the Buczskowsi 2016 field trial.[6]

Four (4) nights of post-treatment snap trapping (aka, "trap outs") occurred at the end of the trial (06/12/17 – 06/15/17), with 35 "victor easy set traps" used in each of the 2 poultry houses. More than 4 nights of snap trapping were originally planned, but manure removal was reportedly scheduled, so snap trapping was discontinued after the 4th night.[7] Snap traps were reportedly "checked for mice after 24 hours and re-set as necessary. The trap-out continued for 7 days and the total number of mice caught was recorded". While capturing residual individuals of the target species in question (house mice) is the most obvious purpose for post-treatment snap trapping, any traps sprung but without capture and/or non-target captures should also be recorded.[8]

Results reported for the trial were poor, with none of the census methods suggesting mouse activity reduction estimates meeting the 70% minimum EPA typically requires. Data for all of the census methods are summarized in the table below.

Activity index	Pre-treatment	Post-treatment	Percent change
Census baiting	2833.77 grams	1932.7 grams	31.8%
Tracking scores*	128	48	62.5%
Live trapping*	46	47	-2.0%
Walking count*	713	492	31.0%
*figures represent total counts rather than means			

Data from the post-treatment snap trapping are provided in the table below.

Post-treatment snap trapping ("trap outs")			
No. trapnights	No. mice caught	No. sprung-no-capture	Trapnight Index
280	209	21	0.8

As the Trapnight Index was far above the 0.1 criterion that is generally used as a "check" against the activity estimates, it is clear that from both it and the activity indices that there continued to be high mouse pressure at the site despite the use of black pearl paste.[9] No non-target captures were reported.

[6] In the Buczkowski 2015 field trial, about 28% toxic bait take was reported. In the Buczkowski 2016 field trial, about 75% toxic bait take was reported.

[7] Given the extremely high numbers of mice captured during the first 4 nights of snap trapping, it is likely that manure removal was considered to be of greater importance to the cooperators than allowing the snap trapping to continue for an additional 3 nights as the treatment was probably judged as having been ineffective at that point.

[8] Generally, in the event of sprung traps with no capture, some adjustment is made to the number of trapnights (e.g., counting a tripped trap as ½ of a trapnight) to account for traps which were no longer able to capture the target animals due to them being temporarily out of commission.

[9] The 0.8 Trapnight Index was derived from the number of mice caught divided by the number of trapnights. As 21 sprung-no-captures were reported, the number of trapnights was adjusted to consider each sprung-no-capture as ½ of a trapnight.

An analysis of the test bait was appended to the back of the report, with a bait manufacture date of "2017-01". An analysis for percent active ingredient is indicated to have occurred on "2019-01" (?), with a reported result of 3.89% alphachloralose having been present. The other ingredients provided on this same form are consistent with the CSF currently proposed for registration.

CONCLUSIONS

These data are considered unacceptable in support of the efficacy data requirement for alphachloralose. Possible reasons for the poor result reported in the field trial include the long history of house mouse problems and rodenticide use at the site, which may have posed too stern a challenge to the bait. Another may be the relatively low amount of toxic bait consumption reported in this trial as compared to the previously reviewed field trials. As a fairly large amount of census bait take was reported (suggesting that the mice were unquestionably present and willing to accept human-supplied food), it seems that the mice at this site were not particularly interested in the bait. In any case, this field trial must be judged a failure.

Donahue, W. (2017) Bait Acceptance and Consumption Evaluations (Efficacy) of Multiple Rodenticide Baits Against Wild Populations of House Mouse, *Mus musculus* Collected from Field Sites in Central California and Brought to the Laboratory for Evaluation. 4p.

No MRID assigned

This report provides a very brief account of laboratory test conducted at "Rainbow Farms", reportedly as a follow-up to the field efficacy trial conducted at the same site.[10] The purpose for the trial was to "evaluate the palatability and efficacy of selected rodent baits (commercial & experimental) using a cage choice test against the house mouse, *Mus musculus*, on wild mice collected from a poultry egg layer facility located in central California". Put differently, this trial represents a comparative test of the alphachloralose bait proposed for registration with several other off-the-shelf, registered rodenticides. It should be noted that EPA has not and would not ever require this type of test to be conducted to support the efficacy data requirement for rodenticides, and further rejects "comparative" efficacy claims made on pesticide labeling.

To summarize, wild house mice were trapped at the site and acclimated to the lab for 10 days, with "dead or weak mice" having been removed from the cages during this time. The mice were placed into "standard laboratory cages" with a surface area of 0.535 ft^2, with 5 mice being assigned to each cage.[11] According to the report, wood shavings were provided as bedding, with water bottles and standard rodent diet (PMI) were provided during the acclimation period. Following that, 7 rodenticide baits were chosen to screen against each of 7 test groups, with each test group/cage containing 5 mice. One untreated control group of 5 mice was also reportedly monitored during the feeding trial. According to the report, "one package or bait or bait block was placed into each of the 7 test cages (one treatment per product with 5 mice) and an untreated control cage." The report only identifies each of the baits by brand name, and in some cases, an abbreviated version of that brand name. As a result, it is not completely clear which products were tested.

[10] As no dates of events are provided in the report aside from a "STUDY COMPLETION DATE" of "14 July 2017", it cannot be stated with certainty exactly when this trial was initiated.

[11] A laboratory choice test using 0.535 ft^2 cages containing 5 mice each raises concerns about basic husbandry, increased mouse interactions (e.g., aggression, defense of preferred cage locations), and the ability of the researchers to properly perform dietary weigh backs due to the increased potential for food items to become heavily soiled. While what might be called "standard laboratory cages" may be appropriate for drug testing, rodenticide choice tests are really *behavior* trials, calling for larger cage sizes in order to minimize the aforementioned variables, among others.

Tomcat Place Pacs
Tomcat Bait Block Hombre Blocks
Black Pearl Paste + Peanut Butter
Rampage Meal Bait
Black Pearl Paste (alphachoralose)
Jaguar Bait Chunx

The report indicates no analyses for percent active ingredient for any of these baits, nor raw batch data for either of the alphachloralose baits.

The "choice" provided to test mice in this trial was between one of the 7 rodenticides and the (presumably) PMI laboratory diet.[12] Replenishment of water and/or food items is not described in the report, nor is any information related to consumption (i.e., weigh backs), which do not appear to have been measured.

The results of this trial are provided in the table below.

Bait	1-week mortality (%)	2-week mortality (%)	3-week mortality (%)
Tomcat Place Pac	0	20	40
Tomcat Bait Bloc	20	20	20
Hombre Blocs	0	0	0
Black Pearl + Peanut Butter	20	40	40
Rampage Meal Bait	20	20	40
Black Pearl Paste	0	40	60
Jaguar Bait Chunx	20	60	100
Untreated (control)	0	0	0

Based upon the reported results, only one of the tested rodenticide baits, Jaguar Bait Chux, managed to kill more than 90% of the mice in any of the 7 test groups, and it only did so after a protracted bait exposure period. No mortality was reported for control mice. The author speculated that this poor result "[indicate] that a more robust experimental design is warranted with a greater number of mice and increased replication". This is certainly true, as it is difficult to draw conclusions about product performance based upon 5-mouse test groups.[13] That aside, it should also be noted that the level of detail provided in this report describing methodology was far below the standards of conduct EPA typically requests for efficacy trials submitted to support registration. Without having consumption data, for example, it cannot be determined whether there was an obvious latency to feed on the toxic baits, whether there were marginal feeders, whether some individuals displayed any drastic changes in dietary choice over time, whether mice consumed normal amounts of the laboratory diet, etc.[14] Without raw entries documenting test subject weight gain/loss and behavioral symptoms, it is not clear whether the subjects, both the ones which survived and the ones which ultimately perished, showed any obvious symptoms of rodenticide poisoning (e.g., bleeding, ataxia) and whether weight gain/loss occurred during the trial. Put simply, there are far too many unknowns for much of anything to be said about

[12] In EPA's former laboratory in Beltsville, MD, laboratory diet was found to be the *least* palatable of the dozen or so diets which were tested as potential challenge diets. When laboratory diet is used as a challenge diet, EPA's experience with it has been that it tends to artificially inflate consumption and mortality of rodenticide baits against which it is tested.

[13] The individual variability in how mice respond to rodenticide efficacy tests would be expected to be amplified considerably with the small sample groups such as the ones used in this trial.

[14] While these data are inherently interesting for a variety of reasons (including those that may affect how the baits are applied in situ), they are of particular importance in linking mortality with rodenticide consumption. The lack of consumption data alone would render this trial unacceptable to support EPA registration.

these data other than that for a small number of wild-collected house mice exposed to these rodenticides under these test conditions, whatever those conditions might have been, all of the baits performed very poorly.

CONCLUSIONS

These laboratory data are considered unacceptable in support of the efficacy data requirement for alphachloralose. As these data could not be used to support EPA's efficacy data requirements regardless of the aforementioned deficiencies, there would be no purpose served by attempting to upgrade the report.

References

Buczkowski, G. (2015). Field evaluation of Black Pearl Paste (alphachloralose) against the house mouse *Mus musculus,* in a confined livestock facility. Unpublished report, Summit Research and Consulting, West Lafayette, IN, 97 pp.

Buczkowski, G. (2016). Field Evaluation of Black Pearl Paste (Alphachloralose) against the House Mouse, *Mus musculus,* in a commercial Equine Facility. Unpublished study prepared by Summit Research and Consulting, LLC. 63p.